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Labile plasma iron and echocardiographic parameters are associated with cardiac events in β-thalassemic patients

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Abstract

Background and Aim: Notwithstanding the improvement in therapies, patients affected by thalassemia major (TM) and intermedia (TI) are still at high risk of cardiac complications. This study aimed at evaluating the incidence and predictive factors for developing cardiac events in adult β-TM and TI patients.

Population and Methods: Data on diagnosis and clinical history were collected retrospectively; prospective data on new-onset cardiac failure and arrhythmias, echocardiographic parameters, biochemical variables including non-transferrin-bound iron (NTBI) and labile plasma iron (LPI), magnetic resonance imaging (MRI) T2* measurement of hepatic and cardiac iron deposits, and iron chelation therapy were recorded during a 6-year follow-up.

Results: Thirty-seven patients, 29 TM and 8 TI, were included. At baseline, 8 TM patients and 1 TI patient had previously experienced a cardiac event (mainly heart failure). All patients were on chelation therapy and only 3 TM patients had mild-to-severe cardiac siderosis. During follow-up, 11 patients (29.7%) experienced a new cardiac event. The occurrence of cardiac events was correlated to high LPI levels (OR 12.0, 95% CI 1.56–92.3, *p* .017), low mean pre-transfusion haemoglobin (OR 0.21, 95% C.I. 0.051–0.761, *p* .21) and echocardiographic parameters suggestive of myocardial hypertrophy. Multivariate analysis disclosed high LPI and left ventricle mass index (LVMI) as independent variables significantly associated with cardiac events. Cardiac iron deposits measured by MRI T2* failed to predict cardiac events.

Abbreviations: BMI, body mass index; Hb, haemoglobin; IQR, interquartile range; IVRT, isovolumetric relaxation time; LPI, labile plasma iron; IVS, interventricular septal thickness; LV, left ventricle; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; LVMI, left ventricle mass index; MRI, magnetic resonance imaging; NTBI, non-transferrin-bound iron; PAPs, systolic pulmonary artery pressure; PWT, posterior wall thickness; SITE, Italian Society of Thalassemia and Hemoglobinopathies; TDTI, transfusion-dependent TI; TI, thalassemia intermedia; TIF, Thalassemia International Federation; TM, β-thalassemia major.

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Conclusion: LPI, Hb levels and echocardiographic parameters assessing cardiac remodelling are associated with cardiac events in adult TM and TI patients. LPI might represent both a prognostic marker and a potential target for novel treatment strategies. Further studies are warranted to confirm our findings on larger populations.

KEYWORDS
anaemia, cardiac complications, iron, labile plasma iron, myocardial hypertrophy, non-transferrin-bound iron, thalassemia

1 | INTRODUCTION

Until the 1970s, β-thalassemia major (TM) had been invariably associated with a poor prognosis early in life.[1,2] The introduction of novel treatment strategies such as optimized transfusion schedules, iron chelation and bone marrow transplantation has had a significant impact on patients' prognosis, with remarkable improvements in survival and quality of life.[1–3] Nevertheless, cardiac complications still represent the main cause of death in TM.[4,5] Similar observations apply to thalassemia intermedia (TI), particularly the transfusion-dependent phenotype (TDTI). Heart siderosis, myocardial hypertrophy and other structural effects of chronic anaemia, alone or in combination, contribute to cardiac pathology in both TM and TI.[6] In TM and TDTI, iron overload is mainly caused by chronic transfusion therapy leading to ventricular (systolic and diastolic) dysfunction and congestive heart failure. On the other hand, in TI, where iron overload is largely due to increased intestinal adsorption, cardiovascular involvement consists mainly of pulmonary arterial hypertension and increased predisposition to thrombosis, stroke and pulmonary embolism, with a largely preserved systolic left ventricular function.[7] Non-transferrin-bound iron (NTBI), a heterogeneous group of circulating iron complexes which are generated almost exclusively under pathological conditions, with potentially toxic effects,[8,9] is usually detectable in thalassemic patients. Labile plasma iron (LPI), a redox-active and chelatable component of NTBI, seems to have a higher propensity to generate reactive oxygen species and cellular damage,[10] thus representing a potential prognostic marker[11] and therapeutic target in TM.[12] There is increasing evidence of a direct role of NTBI and/or LPI in the development of cardiac events in iron-overload conditions,[13–15] although further studies are needed to better clarify the underlying mechanisms. The aim of our study was to identify clinical, biochemical and echocardiographic parameters predictive of cardiac events in patients affected by TM and TI during a six-years follow-up, focusing on the presence and levels of NTBI and/or LPI and echocardiographic parameters.

2 | MATERIALS AND METHODS

Subjects affected by TM or TI, followed at the Centre for Hereditary Anemias, University Hospital of Modena, were evaluated for inclusion in the study. Thirty-seven patients (29 TM and 8 TI) were included in the study, which comprised a retrospective part, in which data such as patient history, comorbidities and previous cardiac events were collected from medical records, and a prospective part, from 2011 to 2016, in which all patients underwent periodical clinical, biochemical and instrumental assessments, as recommended by the Thalassemia International Federation (TIF), and the Italian Society of Thalassemia and Hemoglobinopathies (SITE)[16–18] guidelines. Blood tests, including haemoglobin (Hb) and complete blood count, liver and kidney function tests and iron serum parameters (ferritin, iron and transferrin saturation) were collected at least every 3 months. Echocardiography was performed: (i) yearly in all asymptomatic patients with no evidence of cardiac structural abnormalities; (ii) every 6–8 months or more frequently in symptomatic or decompensated patients. The evaluation of hepatic and cardiac iron deposits by magnetic resonance imaging (MRI) was performed: (i) every 2 years in asymptomatic patients with no evidence of iron overload; (ii) yearly in patients with demonstrated liver or cardiac siderosis; (iii) more frequently (every 6–8 months) in patients with severe cardiac iron overload and clinical signs or symptoms of cardiac decompensation. Hepatic and cardiac iron were quantified according to the T2* algorithm described previously and expressed as ms (heart normal level > 20 ms, liver normal level > 6.4 ms).[19] Patients who developed cardiac events during follow-up and event-free patients were compared, according to clinical, biochemical, MRI and echocardiographic features. We defined a 'cardiac event' as any occurrence of heart failure and/or arrhythmias. Diagnosis of heart failure was based on the occurrence of dyspnea at rest or during exercise or other signs/symptoms consistent with heart failure, together with echocardiographic evidence of left ventricular dysfunction.[20] Diagnosis of

arrhythmia was based on electrocardiographic evidence of arrhythmia (24-h Holter monitoring or standard 12-lead recording). Arrhythmias included atrial fibrillation, defined as an arrhythmia arising from the atrium with an atrial rate > 300 bpm and an irregular ventricular response lasting more than 30 seconds, and supraventricular tachycardia, defined as a tachycardia arising from supraventricular tissue other than atrial fibrillation or flutter.[21]

2.1 | Echocardiographic evaluation

All examinations were performed with the patient in the left-lateral decubitus position, using a General Electronics vivid-3 echocardiogram machine equipped with 2.5–3.5 MHz transducers. Evaluation of cardiac function was made using 2D-echocardio-graphy and intracardiac blood flow was assessed using Doppler colour flow imaging. All values were indexed for body surface area and included: left ventricle end-systolic diameter (LVESD, mm/m^2), left ventricle end-diastolic diameter (LVEDD, mm/m^2), interventricular septal thickness (IVS, mm) and posterior wall thickness (PWT, mm), all measured by M-mode according to ASE recommendations in force at the time of our study; left ventricle mass index (LVMI, g/m^2); left atrial volume (ml/m^2); ejection fraction (EF, %) calculated by Simpson's method; aortic and tricuspid flow velocity (m/s); systolic pulmonary artery pressure (PAPs, mmHg), calculated from tricuspid regurgitation peak. An echocardiographic diagnosis of pulmonary hypertension was made when PAPs were higher than 30 mmHg. LV diastolic dysfunction was diagnosed by evaluating and integrating multiple echocardiographic parameters such as early diastolic wave (E wave), late diastolic wave (A wave), deceleration time of E wave (DT), isovolumetric relaxation time (IVRT) and E wave to A wave (E/A) ratio; LV diastolic dysfunction was classified in four stages according to 2009 ASE recommendations (I = impaired relaxation; II = pseudo-normal; III = restrictive reversible; IV = restrictive non-reversible).

2.2 | NTBI and LPI measurement

In a subset of 23 patients, serum non-transferrin bound iron (NTBI) and labile plasma iron (LPI) were dosed with specific fluorescence-based assays. NTBI was determined by FeROS™ eLPI Kit (Aferrix Ltd., Israel), and LPI was measured by FeROS™ LPI Kit (Aferrix Ltd., Israel).[22] Values were interpreted according to the producer instructions (TSL902 V.11): LPI-negative (normal range), <0.2 LPI units; positive, ≥0.2 LPI units. As for NTBI (TSL904 V.6): negative (normal range), <0.2 eLPI units; positive, >0.2 eLPI units.

2.3 | Statistical analysis

Data were expressed as mean ± standard deviation if normally distributed, median and interquartile range (IQR) if not normally distributed for continuous variables or as a percentage for categorical variables. Chi-square test or Fisher's exact test was used to compare the prevalence of categorical variables between groups. Comparison of different continuous variables between groups was made using Student's t-test or the Mann–Whitney's U test, when appropriate. Simple and multiple logistic regression analysis was used to assess the association between different parameters and the occurrence of cardiac events throughout the chosen time period. Survival analysis and Kaplan–Meier curves were used to calculate the difference in event-free time according to certain variables. Variables that were found significantly associated with the occurrence of cardiac events in the univariate analysis were included in the multivariate analysis, building different models in order to avoid collinearity or excessive loss of cases due to missing data. In all statistical evaluations, a p-value <.05 was chosen as significant. Statistical analysis was performed using SPSS® (v.24.0, Chicago, IL, USA) and STATA® (v. 14.0 College Station, TX: Stata Corp LP, USA) statistical software.[23]

3 | RESULTS

3.1 | Baseline characteristics

Table 1 shows the baseline characteristics of the study population: 29 out of 37 patients (78%) had TM and 8 (21%) had TI; 19 (51%) were females and 18 (48%) males, with a mean age of 41.68 ± 9.19 (range 24–61 years) and a body mass index (BMI) within the normal range; 59.5% had been previously splenectomized and most of them had already developed an endocrinological disorder such as hypogonadism (54%), osteopenia (46%), osteoporosis (43%), hypothyroidism (27%) and type 2 diabetes (16%). In particular, patients with TM developed hypogonadism and hypothyroidism more frequently than patients with TI (70% vs 0, p = .001, 34.5% vs 0, p = .058 respectively). The majority of patients (89%) were on a chronic transfusion regimen, and all TI patients except one were on iron chelating therapy at the beginning of the study: most patients were treated with an oral iron chelator, either Deferasirox (35.1%) or Deferiprone (29.7%); 16.2% of patients were treated with Deferoxamine and 16.2% with the association of Deferoxamine and Deferiprone (Table 1). Therapeutic compliance, as self-reported by patients, was at least equal to 80% in more than 65% of patients, whilst it was inadequate (intake/self-administration of prescribed

TABLE 1 Baseline characteristics of the study population

	TM	TI		Total
	(*n* = 29)	(*n* = 8)	*p*	(*n* = 37)
Male sex, *n* (%)	13 (44.8)	5 (62.5)	.783	18 (48.6)
Age (years)	39.51 ± 7.22	49.5 ± 11.57	.004	41.68 ± 9.16
Ethnicity				
Caucasian/Other, *n* (%)	29 (100)/0	7 (87)/1 (13)	.216	36 (97)/1 (3)
BMI (kg/m^2)	22.94 ± 3.212	22.53 ± 1.08	.726	22.85 ± 2.8
Splenectomized, *n* (%)	16 (55.1)	2 (25)	.312	22 (59.5)
Cardiovascular disease, *n* (%)				
Hypertension	2 (6.9)	0	.61	2 (5.4)
Ischaemic cardiopathy	0	0		0
Endocrinopathy, *n* (%)				
Hypogonadism	20 (69)	0	**.001**	20 (54.1)
Hypothyroidism	10 (34.5)	0	.058	10 (27)
Type 2 diabetes mellitus	5 (17.2)	1 (12.5)	.613	6 (16.2)
Hypoparathyroidism	5 (17.2)	0	.272	5 (13.5)
GH insufficiency	3 (10.3)	0	.470	3 (8.1)
IGF1 insufficiency	3 (10.3)	0	.470	3 (8.1)
Osteopenia	13 (44.8)	4 (50)	.553	17 (45.9)
Osteoporosis	13 (44.8)	3 (37.5)	.517	16 (43.2)
Chelating therapy, *n* (%)				
DFO	6 (20.7)	0	.204	6 (16.2)
DFP	9 (31)	2 (25)	.5568	11 (29.7)
DFX	8 (27.6)	5 (62.5)	.081	13 (35.1)
DFO + DFP	6 (20.7)	0	.204	6 (16.2)
None	0	1 (12.5)		1 (2.7)
Liver Stiffness (KPa)	5.55 ± 2.17	6.32 ± 2.78	.522	5.72 ± 2.27
Cardiac siderosis				
T2* (ms ± SD)	29.95 ± 11.92	36.55 ± 5.62	.154	31.96 ± 10.13
Absent (>20 ms), *n* (%)	13 (44.8)	7 (87.5)		20 (54)
Mild–Moderate (20–10 ms), *n* (%)	2 (6.8)	0	.68	2 (5.4)
Severe (<10 ms), *n* (%)	1 (3.4)	0		1 (2.7)
NA, *n* (%)	13 (44.8)	1 (12.5)		14 (37.8)
Echo parameters				
EF, %	63.36 ± 4.95	64.25 ± 7.52	.585	63.66 ± 5.58
PAPs, mmHg	28.68 ± 5.86	32.41 ± 8.81	.265	29.7 ± 6.78
LA volume, ml/m^2	35.7 ± 14.42	30.41 ± 8.92	.342	34.29 ± 13.25
LVMI, g/m^2	87.41 ± 29.65	100.92 ± 14.65	.255	90.21 ± 27.61
PW thickness, mm	8.58 ± 1.09	8.31 ± 1.09	.546	8.51 ± 1.03
IVS thickness, mm	8.63 ± 1.16	8.56 ± 1.05	.088	8.61 ± 1.11
LV EDD, mm/m^2	23.94 ± 4.65	24.28 ± 4.82	.870	24.03 ± 4.6
TRV, m/s	2.38 ± 0.35	2.5 ± 0.32	.995	2.41 ± 0.33
DDF, *n* (%)	2 (5.8)	4 (10.8)	.269	6 (16.2)
Grade I/IV	1 (3.4)	0		1 (2.7)
Grade II/IV	2 (6.9)	2 (25)	.281	4 (10.8)
Grade III/IV	1 (3.4)	0		1 (2.7)

TABLE 1 (Continued)

	TM ($n = 29$)	TI ($n = 8$)	p	Total ($n = 37$)
Blood tests				
Hb (g/dL)	9.67 ± .64	8.95 ± 1.28	.160	9.51 ± .86
Platelets (*10^3/μL)	416 ± 201	614 ± 218	.070	495 ± 212
Ferritin (ng/mL)	1069 ± 1284	1089 ± 917	.568	1073 ± 1200
Serum iron (μg/dL)	250 ± 51	201 ± 64	.075	242 ± 56
Transferrin (mg/dL)	252 ± 43	253 ± 76	.972	252 ± 48
TSAT (%)	124 ± 19	102 ± 25	.033	121 ± 21
AST (U/L)	25.89 ± 11.05	30.13 ± 13.46	.369	26.83 ± 11.56
ALT (U/L)	29.93 ± 23.83	27.50 ± 13.94	.786	29.39 ± 21.87
Bilirubin (mg/dL)	1.73 ± 1.37	3.2 ± 1.71	**.012**	2.09 ± 1.57
Direct bilirubin (mg/dL)	.515 ± .26	.49 ± .15	.865	.5120 ± .24
Glycemia (mg/dL)	109 ± 50	83 ± 19	.197	104 ± 47
Creatinine (mg/dL)	.7 ± .16	.58 ± .12	.076	.67 ± .16
INR	1.29 ± .70	1.29 ± .31	.995	1.29 ± .629
aPTT	1.06 ± .17	1.19 ± .15	.093	1.09 ± .18
Cholesterol (mg/dL)	122 ± 30	100 ± 20	.075	117 ± 29
Triglycerides (mg/dL)	113 ± 56	121 ± 53	.766	115 ± 55
GGT (U/L)	23 ± 14	8 ± 23	.481	24 ± 16
Prior cardiac events, *n* (%)				
Total	8 (27.5)	1 (12.5)	.357	9 (24.3)
Heart failure	6 (20.6)	1 (12.5)	.606	7 (18.9)
One episode	5 (17.2)	1 (12.5)		6 (16.2)
More episodes	1 (3.4)	0		1 (2.7)
AF	2 (6.89)	0	.194	2 (5)

Abbreviations: AF, atrial fibrillation; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; DDF, diastolic dysfunction; DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; EDD, end-diastolic diameter; EF, ejection fraction; GGT, gamma glutamyl transpeptidase; GH, growth hormone; Hb, haemoglobin; IGF, insulin-like growth factor; INR, international normalized ratio; IVS, interventricular septum; LA, left atrium; LV, left ventricle; PW, posterior wall; NA, not available; PAPs, systolic pulmonary artery pressure; TI, thalassemia intermedia; TM, thalassemia major; TRV, tricuspid regurgitation velocity; TSAT, transferrin saturation.

therapy <50%) in 21.6%. When looking at cardiac siderosis measured by MRI, the mean T2* was within the normal range (>20 msec) for the majority of TM patients and all TI patients, thereby indicating the absence of appreciable siderosis. Only one TM patient presented severe cardiac siderosis (T2* < 10 msec) and two TM patients had mild–moderate siderosis (10 < T2* < 20 msec). The echocardiographic evaluation did not highlight any difference in EF % values between TM and TI subjects and no patient had a reduced EF at baseline. Higher mean PAPs were observed in TI patients (although the difference was statistically non-significant: 32.41 ± 8.81 vs 28.68 ± 5.86 mmHg, $p = .265$). Left atrial volume and IVS thickness were overall higher in TM, although statistical significance was not reached (possibly due to the small sample size). Echocardiographic findings of diastolic dysfunction were present in 16% of patients. With regards to biochemical parameters, the study population presented the expected features of hemolytic anaemia (mean Hb of 9.51 g/dL), with mainly unconjugated high bilirubin (2.09 mg/dL) and overall high levels of serum iron parameters (serum ferritin 1073 ng/mL, serum iron 242 μg/dL, transferrin saturation 121%); patients with TM tended to have higher serum iron (250 vs 201 μg/dL, $p = .075$) and had higher transferrin saturation (124 vs 102, $p = .033$) whilst patients with TI had significantly higher bilirubin levels (3.2 vs 1.73, $p = .012$). As shown in Table 1, 27% of TM and 12% of TI patients had already experienced a cardiac event before the inclusion in the study (heart failure in 19% and atrial fibrillation in 5% of cases). Such patients were more likely to have had a splenectomy or to suffer from endocrine complications such as diabetes and IGF-1 deficiency (Table S1).

3.2 | Occurrence of cardiac events during follow-up

Eleven patients (7 with TM and 4 with TI), experienced at least one cardiac event during the 6-year follow-up, corresponding to an incidence of 29% and a median annual incidence of two patients per year. Amongst those patients, 4 (10%) patients developed more than one cardiac complication. Incidence of heart failure and arrhythmias was 10% and 24% respectively. Amongst arrhythmias, the most common was atrial fibrillation (19%). Patients who developed cardiac events during the follow-up period were more often males and splenectomized, although the difference did not reach statistical significance (72% vs 37%, $p = .06$ and 81% vs 50%, $p = .07$ respectively); they had more often experienced episodes of heart failure or arrhythmias and other cardiac complications before the inclusion in the study (45% vs 15%, $p = .011$). Patients with and without cardiac events during the follow-up period were also compared in relation to their mean transfusion requirement (number of total transfused units and volumes of packed red blood cells transfused yearly and during each session), but we did not find a statistically significant difference in the overall infused volumes and transfusion schedules between the two groups (Table 2). Biochemical data showed that patients with cardiac events had significantly lower pre-transfusion haemoglobin levels at baseline compared with patients without events (8.8 vs 9.7, $p = .003$), although this difference lost its statistical significance during the follow-up (9.13 vs 9.42, $p = .19$). Average serum ferritin was 1578 ng/mL in patients with cardiac events and 1066 ng/mL in patients without cardiac events ($p = .27$), with a significant difference in levels of serum iron at baseline (204 vs 259 μg/dL, $p = .012$), which was not maintained at the following visits, although a lower trend in patients who decompensated remained (average values 220 vs 263 μg/dL, $p = .074$).

3.3 | Cardiac events according to parenchymal iron overload

When looking at the degree of organ siderosis, both average cardiac and hepatic T2* values were within the normal range but slightly lower in patients with cardiac events (33.01 vs 34.5 ms, $p = .718$ and 10.9 vs 14.71, $p = .35$ respectively), although the difference was not statistically significant. Considering each type of cardiac event separately, average cardiac T2* was lower in patients with heart failure (although still within the normal range) compared with patients with arrhythmias (27.1 vs 37.6 ms, $p = .215$) but the difference was not statistically significant. As to chelation therapy, during the follow-up period, the percentage of patients treated with Deferasirox increased (Figure 1); however, no significant association was found between exposure to iron chelation treatment or type of chelating agent and the incidence of cardiac events (data not shown).

3.4 | Cardiac events according to echocardiographic parameters

As shown in Table 3, the two groups were compared according to echocardiographic parameters: in the 2013–2014 period, PW thickness, IVS thickness and LVMI were significantly increased in patients with cardiac events (9.35 vs 8.41 mm, $p = .009$; 9.55 vs 8.47 mm, $p = .005$; 114.61 vs 81.93, $p = .000$), indicating the presence of myocardial hypertrophy. Ejection fraction, although within the normal range in both groups, was significantly reduced in patients with cardiac events compared with patients without events (57% vs 61%, $p = .001$), and showed a trend toward a further reduction during follow-up, from a starting average value of 63% to an ending average value of 57%. Furthermore, patients with cardiac events had a slightly increased LVESD. During follow-up, signs of diastolic dysfunction were registered in 36% of patients with cardiac events and 26% of patients without events and were more prevalent in patients with heart failure rather than rhythm disturbances, despite the difference did not reach statistical significance ($p = .088$). Additionally, we found a significant increase in LA volume (45.5 vs 31 mL/m^2, $p = .006$), and a non-statistically significant increase in LVEDD, representing signs of early diastolic dysfunction possibly due to reduced ventricular compliance, in patients with cardiac events.

3.5 | NTBI and LPI according to echocardiographic parameters and cardiac events

Non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) were measured in a subgroup of patients (23/37) and, as expected, their values were on average increased (1.259 U and 0.365 U, respectively) compared to normal. Interestingly, both NTBI and LPI were higher in those patients who developed a cardiac event during the whole follow-up (2.028 vs 0.848, $p = .024$ and 0.90 vs 0.079, $p = .02$, respectively; Table 4). Since LPI/NTBI determination was performed only in 2014, we evaluated their correlation with biochemical, echocardiographic and clinical parameters collected around the same time . LPI was found to be positively related to serum ferritin ($p = .037$) and QTc prolongation ($p = .021$); moreover, high LPI correlated with

TABLE 2 Patients' characteristics at baseline according to the development of new cardiac events.

	Patients with cardiac events ($n = 11$)	Patients without cardiac events ($n = 26$)	p
Male/female sex, *n*	8/3	10/16	.060
Age, years	42.64 ± 10.72	41.27 ± 8.62	.684
BMI, kg/m^2	22.88 ± 2.63	22.83 ± 3.02	.964
Splenectomized, *n* (%)	9/2 (81.8)	13/13 (50)	.073
Prior cardiac event, *n* (%)	5 (45)	4 (15)	**.011**
Transfusion requirement			
None	1 (9.9)	3 (11.5)	.747
1 U RBCs	7 (63.6)	13 (50)	
1 U RBCs	3 (27.2)	10 (38.4)	
Transfused volume (mL/kg/day)			
2015	0.304 ± 0.181	0.326 ± 0.181	.751
2016	0.276 ± 0.147	0.320 ± 0.176	.512
Calculated iron intake (mg/kg)			
2015	0.280 ± 0.175	0.326 ± 0.175	.493
2016	0.270 ± 0.136	0.138 ± 0.164	.512
Biochemical parameters			
Pre-transfusion Hb (baseline), g/dl	8.87 ± 1.00	9.76 ± 0.669	**.003**
Pre-transfusion Hb (follow-up) g/dl	9.13 ± .672	9.42 ± .558	.19
Patelets, migl/μL	578 ± 168.11	463 ± 221.57	.147
AST, U/L	32 ± 11.69	24.84 ± 11.09	.096
ALT, U/L	28.5 ± 15.8	29.73 ± 24.05	.882
Total bilirubin, mg/dL	2.19 ± 1.46	2.04 ± 1.64	.805
Glycemia, mg/dL	110 ± 40.95	102.5 ± 49.93	.688
Creatinine, mg/dL	0.67 ± 0.22	0.67 ± 0.13	.992
INR*	1.14 ± 0.14	1.14 ± 0.11	.98
aPTT	1.22 ± 0.23	1.03 ± 0.11	**.001**
Cholesterol, md/dL	121 ± 30.68	116.8 ± 29.6	.073
Triglycerides, mg/dL	115.5 ± 68.2	115.33 ± 52.01	.994
GGT, U/L	34.3 ± 21.05	20.12 ± 12.8	**.02**
Iron serum parameters			
Serum ferritin, ng/mL	1578.18 ± 1622.59	1066 ± 1117.40	.274
Serum ferritin, log	3.00 ± .435	2.90 ± .293	.416
Serum iron (baseline), μg/dL	204 ± 62	259 ± 45	**.012**
Serum iron (follow-up), μg/dL	220 ± 61	263 ± 62	.074
Serum TIBC mg/dL	247.66 ± 52.8	255 ± 47.4	.712
Cardiac T2* (msec)	33.01 ± 12.02	34.54 ± 4.09	.718
Liver T2* >6.3 (msec)	10.90 ± 11.57	14.71 ± 9.86	.350
ECG alteration, *n* (%)			
Total	9 (75)	17 (65.3)	.841
Supraventricular	4 (36.3)	10 (38.4)	.495
Ventricular	4 (36.3)	9 (34.6)	.591
Right bundle branch block	4 (36.3)	6 (54.5)	.683
T wave inversion	2 (7.69)	5 (19.2)	.571

(Continues)

TABLE 2 (Continued)

	Patients with cardiac events ($n = 11$)	Patients without cardiac events ($n = 26$)	p
ST alterations	5 (45.4)	12 (46.1)	.845
Alterations in cardiac frequency	3 (11.5)	3 (11.5)	.178
QTc prolongation	4 (36.6)	2 (7.6)	**.048**

Abbreviations: ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; U, unit(s); ECG, electrocardiography; GGT, gamma glutamyl transpeptidase; Hb, haemoglobin; INR, international normalized ratio; RBCs, red blood cells; TIBC, total iron binding capacity.

*After exclusion of outliers and/or patients on anticoagulation therapy ($n = 3$).

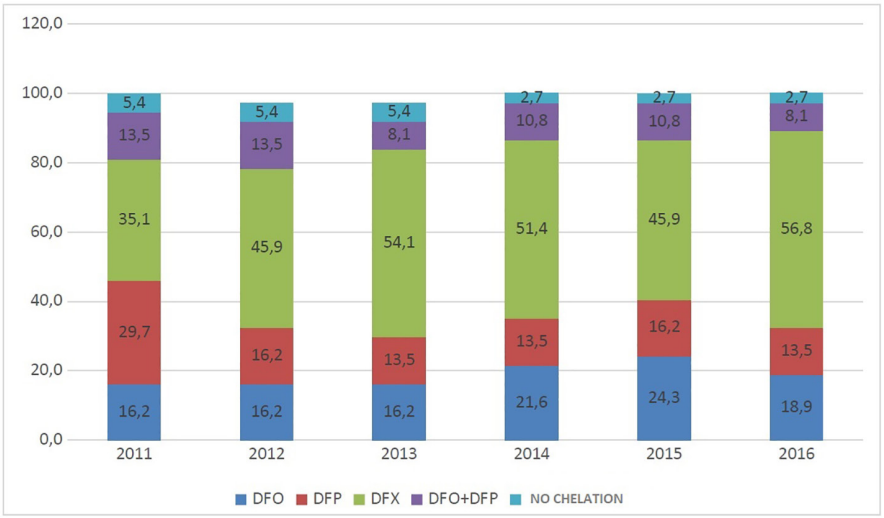

FIGURE 1 Iron chelation therapy during follow-up. DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox.

IVS ($p = .025$), PWT ($p = .024$) and LVMI ($p = .010$) in the subgroup of patients for whom such measurements were available (Table S2).

3.6 | Predictors of cardiac clinical events

To find the best combination of parameters associated with cardiac events, we tested the multivariate analysis different models including those variables which were significantly associated with the outcome of interest in the univariate analysis (Table 5). Therefore, such models included pre-transfusion Hb, high LPI and one echocardiographic parameter amongst LVESD, PWT, IVS, LA volume, EF and LVMI. As explained above, the multivariate models included data collected as close as possible to the year in which the LPI/NTBI determination was performed. In 3 out of 6 models, high LPI was the only parameter associated with cardiac events in our population, even after adjusting for the previous occurrence of cardiac events: OR = 12 (95% CI 1.56–92.29; $p = .017$) when included with pre-transfusion Hb and IVS; OR 19.4 (95% CI 1.74–216.54; $p = .016$) when included with pre-transfusion Hb and EF; OR 9.12 (95% CI 1.15–73.24; $p = .037$) when included with pre-transfusion Hb and LVESD. LVMI was significantly associated with the occurrence of cardiac events in the multivariate model which also included LPI and pre-transfusion Hb: OR 1.14 (95% CI 1.02–1.28). Figure 2 shows the event-free survival of our population according to high or normal LPI levels.

4 | DISCUSSION

Despite the huge improvements in transfusion and iron chelation strategies, patients with β-TM and TI are still at high risk of developing cardiac events—mainly heart failure and arrhythmias. Therefore, there is an urgent need for tools and predictive scores able to identify patients at higher risk. In this regard, several studies have suggested a predictive role of MRI cardiac T2*, whereas others have investigated serum ferritin and liver iron concentration. To date, though, the correlation of these data with clinically significant events has not been demonstrated and high-risk patients are still not identified on time for early and effective intervention.

We preliminarily observed that a high incidence of cardiac events, mainly represented by supraventricular arrhythmias and cardiac failure, could be observed in a

TABLE 3 Echocardiographic parameters according to the development of new cardiac events.

	Patients with cardiac events ($n = 11$)	Patients without cardiac events ($n = 26$)	p
EF (%)			
2011–2012	63.11 ± 4.13	63.87 ± 6.1	.732
2013–2014	58.95 ± 5.15	61.81 ± 3.8	.082
2015–2016	57.05 ± 3.77	61.63 ± 3.25	**.001**
PAPs (mmHg)			
2011–2012	28.28 ± 6.84	30.36 ± 6.88	.516
2013–2014	25.83 ± 7.27	28.44 ± 7.59	.395
2015–2016	26.25 ± 4.9	25.38 ± 7.06	.729
LA volume (mL/m^2)			
2011–2012	31.37 ± 7.73	35.54 ± 15	.439
2013–2014	45.50 ± 12.7	31.00 ± 6.64	**.006**
2015–2016	33.47 ± 24.6	25.39 ± 11.72	.343
LVMI (g/m^2)			
2011–2012	92.68 ± 17.72	76.79 ± 22.63	.18
2013–2014	114.61 ± 23.68	81.93 ± 15.81	**.000**
2015–2016	104.45 ± 25.91	81.68 ± 12.20	**.002**
PW thickness (mm)			
2011–2012	8.72 ± 1.17	8.43 ± 1.06	.508
2013–2014	9.35 ± 0.783	8.41 ± 0.925	**.009**
2015–2016	8.75 ± 0.978	8.34 ± 0.789	.220
IVS thickness (mm)			
2011–2012	9.00 ± 1.17	8.45 ± 1.07	.222
2013–2014	9.55 ± 1.06	8.47 ± 0.872	**.005**
2015–2016	8.95 ± 1.03	8.39 ± 0.797	.102
LV EDD (mm/m^2)			
2011–2012	22.93 ± 6.02	24.52 ± 3.92	.428
2013–2014	32.20 ± 4.66	30.02 ± 4.42	.210
2015–2016	31.80 ± 3.85	30.26 ± .695	.254
LV ESD (mm/m^2)			
2013–2014	20.50 ± 3.46	17.72 ± 3.33	**.047**
2015–2016	17.30 ± 6.46	14.89 ± 6.1	.349
DDF *n* (%)	4 (36.3)	7 (26.9)	.420

Abbreviations: Pts, patients; DDF, diastolic dysfunction; EF, ejection fraction; PAPs, systolic pulmonary artery pressure; LA, left atrium; LV, left ventricle; LVMI, left ventricle mass index; PW, posterior wall; IVS, interventricular septum; EDD, end-diastolic diameter; ESD, end-systolic diameter.

TABLE 4 NTBI and LPI values according to the development of new cardiac events

	Patients with cardiac events ($n = 8$)	Patients without cardiac events ($n = 15$)	p	Total ($n = 23$)
NTBI (U)				
% abnormal values	7 (87%)	11 (73%)	.414	18 (78%)
Mean ± SD	2.028 ± 1.377	0.848 ± 0.949	**.024**	1.259 ± 1.227
LPI (U)				
% abnormal values	6 (75%)	3 (20%)	**.017**	9 (39%)
Mean ± SD	0.900 ± 0.921	0.079 ± 0.1162	**.002**	0.365 ± 0.662

Abbreviations: LPI, labile pool iron; NTBI, non-transferrin bound iron; SD, standard deviation.

TABLE 5 Univariate and multivariate analysis for correlation with the risk of cardiac events.

	Univariate regression analysis		Multivariate regression analysis	
Variable	**OR (95% CI)**	***p***		
LPI	12 (1.5–92.2)	.02		
Pre-transfusion Hb (baseline)	0.21 (0.05–0.76)	.02		
Prolonged QTc	7.33 (1.07–50.1)	.04		
Age		ns		
Sex		ns		
Serum iron		ns		
GGT		ns		
			Model 1	**OR (95% CI)**
IVS	4.12 (1.27–13.7)	.02	IVS	ns
			High LPI	12 (1.56–92.29)
			Pre-transfusion Hb	ns
			Previous cardiac events	ns
			Model 2	**OR (95% CI)**
LVESD	1.26 (0.98–1.62)	.06	LVESD	ns
			High LPI	9.12 (1.15–73.24)
			Pre-transfusion Hb	ns
			Previous cardiac events	ns
			Model 3	**OR (95% CI)**
EF	0.82 (0.65–1.03)	.09	EF	ns
			High LPI	19.4 (1.74–216.54)
			Pre-transfusion Hb	ns
			Previous cardiac events	ns
			Model 4	**OR (95% CI)**
LVMI	1.114 (1.032–1.203)	.005	LVMI	1.146 (1.025–1.281)
			High LPI	ns
			Pre-transfusion Hb	ns
			Previous cardiac events	ns
			Model 5	**OR (95% CI)**
PWT	1.09 (0.93–1.31)	.22	PWT	ns
			High LPI	ns
			Pre-transfusion Hb	ns
			Previous cardiac events	ns
			Model 6	**OR (95% CI)**
LAV	1.22 (1.05–1.41)	.01	LAV	ns
			High LPI	ns
			Pre-transfusion Hb	ns
			Previous cardiac events	ns

Abbreviations: CI, confidence interval; EF, ejection fraction; Hb haemoglobin; IVS, interventricular septum; LAV, left atrium volume; LPI, labile plasma iron; LVESD, left ventricle end-systolic diameter; LVMI, left ventricle mass index; ns, not significant; OR, odds ratio; PWT, posterior wall thickness.

population of adequately chelated patients with normal heart T2* values, suggesting that factors other than cardiac siderosis could lead to cardiac dysfunction. Therefore, we analysed clinical, biochemical, radiological and echocardiographic parameters possibly associated with cardiac complications. We decided to put together the development of arrhythmias or heart failure as 'cardiac events' not only to increase the number of outcomes but also because they are cardiac complications of thalassemia that can equally affect the quality of life and prognosis of patients.

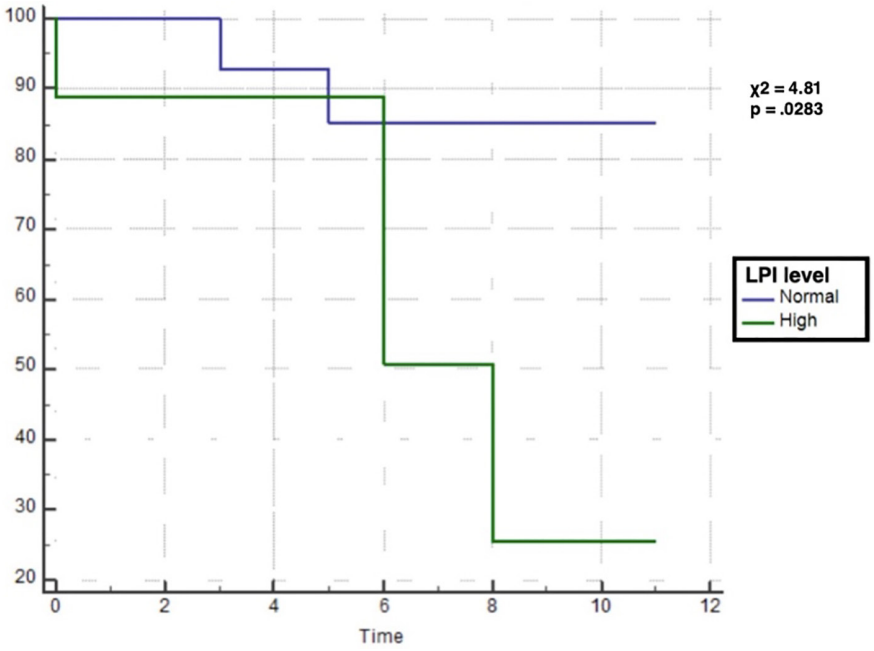

FIGURE 2 Cardiac event-free survival of the study population according to LPI.

As expected, a new cardiac event was more likely to occur in subjects who had already experienced a cardiac complication. Additionally, the proportion of patients who experienced a cardiac event during follow-up was higher in the TM than in the TI group (50% vs 26%, $p = .2$), although this difference did not reach statistical significance.

Interestingly, patients with cardiac events also showed a statistically significant alteration in coagulation parameters (PT and aPTT), possibly an expression of the hypercoagulable state related to the abnormalities in the red blood cell surface, platelet activation and endothelial cell activation.[24]

Furthermore, patients with cardiac events had a significantly lower EF as compared to patients without events, even if within the normal range. However, it must be noticed that the EF normal range has been set in non-anaemic patients, and it is under debate whether it should be applied to patients with thalassemia. A slight increase of LVESD was also found in this group of patients, possibly indicating a subtle alteration of systolic function.

A diastolic, rather than systolic dysfunction was common in patients with cardiac events and was significantly associated with heart failure but not with rhythm disturbances. Early identification of diastolic failure is therefore of great importance and should be consistently addressed with new techniques, such as tissue Doppler imaging (TDI) strain, strain rate and longitudinal deformation.

Patients who developed cardiac events had significantly increased IVS, PW thickness and LVMI, reflecting myocardial hypertrophy secondary to high cardiac output and chronic volume overload—both related to chronic anaemia. Actually, pre-transfusion haemoglobin at baseline was significantly lower in patients who had cardiac events during follow-up. The transfusion schedule did not seem to affect cardiac risk in our population of transfusion-dependent patients, nor did the type of chelator and the period of exposure to the chelating agent during the follow-up. Average serum ferritin was higher in patients with cardiac events, but the difference did not reach statistical significance, likely due to the relatively low number of patients and the high variability of this parameter within groups.

More interestingly, labile plasma iron (LPI) positively correlated to the onset of cardiac events. Together with LVMI, LPI remained the only variable associated with the development of cardiac events in multivariate analysis. LVMI was significantly different in the two populations even after adjustment for high LPI, baseline pre-transfusion Hb and the previous occurrence of cardiac events in the multivariate model. The fact that LPI lost significance when compared with LVMI at multivariate analysis might be due to the combination of the high statistical significance at the univariate analysis of IVS and PWT (which contribute to determine the LVMI) on a small sample size. LPI, the most reactive and readily chelatable fraction of NBTI, appears in the bloodstream only under pathological conditions and was previously reported to be elevated in thalassemic populations and related to the degree of iron overload and the chelation regimen,[25] but to date, no studies have addressed its potential role in predicting clinical events. In our study, LPI also correlated to QTc prolongation and serum ferritin levels but was not influenced by the exposure to chelating agents. Moreover, it was superior to serum ferritin in predicting cardiac events,

suggesting that LPI may play a pathogenic role in cardiac dysfunction and not be a simple marker of iron excess. In accordance with this, LPI has been hypothesized to directly infiltrate cells via transferrin receptor-independent mechanisms (DMT1, ZIP14, light-calcium channels, endocytosis).[26]

Altogether, these findings suggest that the combined effect of anaemia, hemolysis and pro-oxidant iron species, rather than the extent of tissue iron deposits per se, may be responsible of cardiac damage in thalassemic patients. As to systolic function and cardiac remodelling, our data are consistent with previous observations showing a relation between lower EF and increased cardiac output in response to chronic anaemia—the latter causing hyperdynamic circulation, decreased blood viscosity and vasodilation.[27] Besides this, we postulate that chronic hemolysis, as a consequence of ineffective erythropoiesis, is responsible for the activation of platelets, endothelium and the coagulation cascade, as well as the appearance of potentially toxic iron species in the bloodstream, such as LPI, which may be directly responsible for cardiac oxidative damage. The diastolic function may be primarily affected by oxidative damage, therefore early identification of diastolic failure is of great importance, and should be addressed consistently with new techniques, such as tissue Doppler imaging (TDI) strain, strain rate and longitudinal deformation.

Our study is limited by the small sample size and the high number of patients with previous cardiac events (a high-risk subgroup per se). For cost/benefit reasons, we could not include baseline T2* cardiac MRI for all patients, as in our centre this evaluation is performed every 2 years, in the absence of cardiac siderosis and according to SITE guidelines. Nevertheless, the strengths of our study are represented by the detailed clinical, biochemical and echocardiographic characterization of the patients and the prolonged time of observation, which allowed important clinical correlations. In fact, there is a vast amount of medical literature about echocardiographic findings in thalassemia, but this is the first study, to the best of our knowledge, which addresses their potential association with the risk of developing clinical events. Similarly, previous studies found LPI to be related to cardiac siderosis or structural and functional myocardial alterations but did not investigate its correlation with clinical events. Further investigations are warranted to confirm our findings on larger populations of thalassemic patients.

In conclusion, echocardiography remains of prominent importance in identifying thalassemic patients at risk of cardiac complications; new echocardiographic techniques need to be implemented to detect preclinical diastolic and systolic dysfunction, especially in patients without overt cardiac iron deposits. The roles of NTBI and LPI as prognostic tools and new therapeutic targets in thalassemia need to be validated in larger prospective studies.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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